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Anti-MRSA activity of Brown and Red algae from Gulf of Mannar Coast, South India

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ABSTRACT:
Phytochemical analyses and *in vitro* antibacterial activity of different organic solvents with increasing polarity *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *Sargassum wightii*, *Stoechospermum marginatum*, *Gracilaria edulis*, and *G. verrucosa* against *Staphylococcus aureus* (MTCC 737 & 7443), and three clinical isolates of MRSA were tested and the extent of inhibitory zone, Minimum inhibitory concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were determined. The ethyl acetate extracts of the seaweeds showed the presence of Phytochemicals, terpenoids, tannins, phenolic compounds and steroids strongly than the other solvent extracts. The highest activity was recorded in the ethyl acetate extract of *S. marginatum* than the other extract tested. The mean zone of inhibition produced by the extracts in disc diffusion assays against the tested bacterial strains ranged from 7.1 to 21.5 mm. The lowest MIC (62.5 µg/ml) and MBC (125 µg/ml) values were observed in the ethyl acetate extract of *S. marginatum* against *S. aureus* (737 & 7443), MRSA1 and MRSA3. Further separation of active principle from the potential seaweed extract as a source of antibacterial compound useful for the control of Methicillin resistant *S. aureus* is under progress.
Key words: Marine Macro Algae, MRSA, Disc diffusion method, MIC and MBC

INTRODUCTION

In developing countries, bacterial infections are widespread, especially in informal settlements, due to poor sanitation and unhygienic conditions. Furthermore, diseases such as AIDS, malaria and tuberculosis result in higher morbidity and mortality than those caused by susceptible pathogens the global impact of increasing resistance is a major concern [1]. The coagulase-positive *Staphylococcus* species *S. aureus* is a major cause of many serious hospital- and community acquired infections. It can colonise the skin and anterior nares of individuals and is carried by a significant proportion of the population. *S. aureus* has a wide range of virulence factors and can cause infections at many anatomical sites. The most common infections are those of the skin and soft tissues, including cellulitis, impetigo and soft tissue abscesses, the latter being frequent in diabetic patients [2]. Since methicillin was introduced in 1959 to resolve infections caused by penicillin-resistant *S. aureus*, the incidence of methicillin resistance in Staphylococci has increased rapidly [3].

Methicillin-resistant *S. aureus* (MRSA) is a major cause of infections in healthcare institutions [4] and more recently in the community [5]. MRSA was first reported in 1961, two years after the introduction of methicillin for the treatment of penicillin-resistant *S. aureus* infections [6]. Despite extensive infection control efforts, methicillin resistance among isolates of *S. aureus* has steadily increased. Data from the National Healthcare-associated Infections Surveillance (NHIS) system of the Centers for Disease Control and Prevention show that 50% of healthcare-associated *S. aureus* isolates are now resistant to methicillins [7]. The resistance has increased relentlessly and well recognized as a global nosocomial problem in recent years. MRSA is resistant to β-lactams, amino glycosides, fluoroquinolones and macrolide, only sensitive to vancomycin, but the isolate of vancomycin-resistant *S. aureus* (VRSA) has also been reported. It is resulted from the selective pressure of antibiotics currently used, leading to high morbidity and mortality [8].

The discovery, development and clinical use of antibiotics during the nineteenth century have substantially decreased public health hazards resulting from bacterial infections. However, there has been a parallel and alarming increase in bacterial resistance to existing chemotherapeutic agents as a result of their injudicious use [9]. Antibiotics provide a main base for the therapy of microbial infections. Since, the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi drug resistant strains of several groups of microorganisms [10].

Marine macroalgae are commonly referred as seaweeds and are classified as green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae) algae depending on their nutrients and chemical composition [11]. Brown algae are represented by about 265 genera and over 1500 species of which about 99.7% are marine and rhodophyceae, commonly called red algae, is represented by approximately 4000 species, about 98% of which are marine[12]. Brown algae contain a wide variety of acid polysaccharides such as the alginic acid, consisting exclusively of uranic acid the homo fucans and the hetero fucans that contain portion of neutral sugar and uranic acid in addition to sulphated fucose [13]. Red algae have been used since ancient times as food, fodder and fertilizer and as sources of medicinal drugs. Today seaweeds are used as the raw materials for industrial production of agar,

carrageenan and alginates [14], but they continue to be widely consumed as food in Asian countries [15].

Hence, the present work was aimed to screen and evaluate the efficiency of hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. wightii, S. marginatum, G. edulis,* and *G. verrucosa* as antibacterial agents against the MRSA.

MATERIALS AND METHODS

Sample collection

Sargassum wightii, Stoechospermum marginatum, (Phaeophyceae) *Gracilaria verrucosa* and *Gracilaria edulis* (Rhodophyceae) were collected from Manappad (Lat. 8°30′N; Long. 78°8′E), Tuticorin district, the Gulf of Mannar Marine biosphere, Tamil Nadu, India. The collections were made from the months of November to January, 2012. The algae were identified by Dr. R. Selvaraj, Former Professor of Botany, Annamalai University and the museum specimens are deposited in the Department of Botany, Annamalai University.

Preparation of Extracts

The algal species were handpicked during low tide and washed thoroughly with sea water to remove all unwanted impurities, epiphytes, animal casting, and adhering sand particles etc. Morphologically distinct thallus of algae were placed separately in new polythene bags and were kept in a ice box containing slush ice and transported to the laboratory. Then, the samples were blotted and dried using sterile tissue paper. The shade dried samples were again cleaned with sterile distilled water to remove the remaining salt on the surface of the samples to avoid pumping of the solvent during the extraction process. The algal samples were shade dried followed by oven drying at 50 °C for an hour and milled in an electrical blender. Five hundred grams of powdered samples were packed in Soxhlet apparatus and extracted with different solvents like hexane, chloroform, ethyl acetate, acetone and methanol for 72 hours. The extracts were pooled and the solvent were evaporated under vacuum in rotary evaporator (Heidolph, Germany) at 4 °C and the dried extracts were stored at 4 °C in refrigerator for antibacterial assay.

Phytochemical screening

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. marginatum*, *S. wightii*, *G. verrucosa* and *G. edulis* were used for qualitative phytochemical studies. Phytochemicals like Terpenoids, Tannins, Cardic glycosides, Steroids, Alkaloids, Phenolic compounds, Coumarins, and Diterpenoids were carried out according to the method described by Harborne [16] and Trease and Evans [17].

Bacterial strains used

The bacterial strains *viz.,* of *Staphylococcus aureus* (MTCC 737 & 7443), were procured from Microbial Type Culture Collection (MTCC), Chandigarh. Three clinical isolates of MRSA strains were obtained from Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai nagar, Tamil Nadu, India. The stock cultures were maintained on nutrient agar (Hi-MediaM087) medium at 4 ºC. *In vitro* antibacterial activity was determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) for *S. aureus* (MTCC 7443) and MHA and MHB supplemented with 4% sodium chloride for MRSA. The media were obtained from Himedia, Mumbai.

Antibiotic sensitivity test

Antibiotic sensitivity of the bacterial strains were determined by standard method [18] using antibiotics *viz.*, Methicilin (ME 5 µg/disc), Oxacillin(OX µg/disc), Linezolid (LIN 30 µg/disc), Vancomycin (VAN 30 µg/disc) Amikacin (AK 30 µg/disc), Antibacterial agents from different classes of antibiotics Ampicillin (AMP 10 µg/disc), Cefixime (CFM 5 µg/disc), Ceftazidime (CAZ 30 µg/disc), Ciprofloxacin (CIP 5 µg/disc), Chloramphenicol (C 30 µg/disc), Erythromycin (E 15 µg/disc), Gentamycin (GEN 10 µg/disc), Norfloxacin (NX 10 µg/disc), Nalidixic acid (NA 30 µg/disc), Ofloxacin (OF 5µg/disc), Streptomycin (S 10 µg/disc) and Tetracycline (TE 30 µg/disc) (Himedia, Mumbai).

Detection of MRSA

Three isolates of MRSA were analyzed and confirmed by Gram's stain and conventional biochemical methods viz., gram stain, catalase test, mannitol test and coagulase test [19]. Methicillin resistance was detected by disc diffusion technique [20] using Oxacillin 1 µg/disc. Retesting was done using Methicillin 5 µg/disc. Zone of inhibition less than 10 mm or any discernible growth within the zone of inhibition was the indication of methicillin resistance**.**

Antibacterial assay:

Inhibition Zone determination by Disc diffusion assay

The agar diffusion method [20] was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 ml of MHA for *S. aureus* (MTCC 7443) and MHA (Hi-MediaM173) supplemented with 4 % sodium chloride for MRSA and allowed to solidify. MHA plates were inoculated by streaking the swab over the entire agar surface using bacterial suspensions containing 10^8 colony forming units (CFU) per ml and allowed to dry for 10 minutes. The crude extracts were dissolved in 10% DMSO (Hi-MediaRM5856) and under aseptic conditions; sterile discs were impregnated with 20 µl

of different concentrations of extracts (500, 250, 125 µg/ml). The discs with extracts were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Methicillin (5 µg/disc) was used as positive control and 10 per cent DMSO was used as blind control in these assays. Finally, the inoculated plates were incubated at 37 ℃ for 24 h *S. aureus* (MTCC 7443), 35 ℃ for 24 - 48 h (MRSA). The zone of inhibition was observed and measured in millimeters. Each assay in this experiment was repeated three times.

Determination of the Minimum inhibitory concentration (MIC)

The MIC of the crude extracts, a modified resazurin microtitre plate assay was used as reported by Sarker *et al.* [21] Sterile MHB (Hi-MediaM391) for *S. aureus* (MTCC 7443) and MHA supplemented with 4% sodium chloride for MRSA and was used in this assay. 50 µl of respective broth was transferred in to each well of a sterile 96-well micro titer plate (Hi-Media TPG 96). The plant extracts was dissolved in 10 per cent DMSO to obtain 2000 µg/ml stock solution. 50 µl of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth 50 µl of the solution was transferred to the second well and in this way, the dilution procedure was continued to a twofold dilution to obtain concentrations like 1000 to 15.625 µg/ml of the extract in each well. To each well, 10 µl of resazurin indicator solution was added. (The resazurin (Hi-MediaRM125) solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water). Then 30 µl of Sterile MHB for *S. aureus* (MTCC 7443) and MHA supplemented with 4% sodium chloride for MRSA was added to each well. Finally, 10 µl of bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 CFU/ml. Each plate had a set of controls: a column with all solutions with the exception of the crude extracts; a column with all solutions with the exception of the bacterial solution adding 10 µl of MHB instead and a column with 10 % DMSO solution as a negative control. The plates were incubated at 37 ℃ for 24 h *S. aureus* (MTCC 7443), 35 ℃ for 24 - 48 h (MRSA). The colour change was then assessed visually. The growth was indicated by color changes from purple to pink (or colourless). The lowest concentration at which colour change occurred was taken as the MIC value.

Determination of the Minimum Bactericidal Concentration (MBC)

MBC of the extracts were determined by plating a loop full of samples from each MIC assay well with growth inhibition into freshly prepared MHA for *S. aureus* (MTCC 7443) and MHA supplemented with 4% sodium chloride for MRSA. The plates were incubated at 37 ℃ for 24 h *S. aureus* (MTCC 7443), 35 ℃ for 24 - 48 h (MRSA). The MBC values were recorded as the lowest concentration of the extracts that did not permit any visible bacterial growth after the period of incubation.

RESULTS

The antibiotic resistance of bacterial strains of both clinical and standard strains was confirmed by CLSI-M100-2012 method. The standard strains of tested, *S. aureus* (MTCC 7443) were found to be reference drug highly sensitive to all the antibiotics tested except AMP and *S. aureus* (MTCC 737) was found to be highly resistant to all antibiotics tested except GEN, S, TE, AK, E and C. The three clinical isolates of MRSA were highly resistant to all the antibiotics resistance tested except GEN, S, TE, AK, E, C, VAN, LIN, NX, NA, and OF.

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. marginatum S. wightii, G. edulis*, and *G. verrucosa* were used to analyse the Phytochemicals, terpenoids, tannin, cardiac glycosides, steroids, alkaloids, phenolic compound, coumarins and diterpenoids. The ethyl acetate extracts of *S. marginatum*, *S. wightii*, *G. edulis*, and *G. verrucosa* contained presence of Phytochemicals, terpenoids, tannins, phenolic compounds, and steroids than the other solvent extracts. Among the Phytochemicals, cardiac glycosides were present only in chloroform extracts of *S. marginatum* and *S. wightii*. Among the tested phytochemicals, diterpenoids and cardiac glycosides were absent in all the extracts of *G. edulis* and *G. verrucosa*. Alkaloids were present only in the chloroform and ethyl acetate extracts of *G. edulis* and *G. verrucosa*. Alkaloids and coumarins were absent in all the extracts of *S. marginatum* and *S. wightii*.

The different solvents with increasing polarity viz., hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. marginatum, S.wightii, G. verrucosa* and *G. edulis* were tested against three clinical isolates and two standard strains of *S. aureus* (737 &7443). The mean values are presented in (Table 1 to 4). Among the tested extracts, the ethyl acetate possessed notable activity against *S. aureus* (737 &7443) strains tested. The ethyl acetate extract of *S. marginatum* showed promising activity against all the bacterial strains tested. All the extracts of marine macro algae possessed significant antibacterial activity against *S. aureus* (737 &74443) and 3 isolates of MRSA tested when compared to the available antibiotics tested. There was no much variation among the clinical and standard bacterial strains towards the algal extracts tested. When the different extracts were assayed against the test bacteria by disc diffusion assays, the mean zone of inhibition recorded were between 7.1 and 21.5 mm. Methicillin (5 µg/disc) antibacterial positive control produced mean zone of

inhibition ranged from 7.1 to 9.0 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The lowest MIC value of 62.5µg/ml and MBC value of 125µg/ml were recorded in the ethyl acetate extracts of *S. marginatum* against *S. aureus* (737 & 7443), MRSA1 and MRSA3.

Table: 1 Antibacterial activity of *Sargassum wightii* against bacterial strains

Bacteria strains / Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)					
	500	250	125	Methicillin (5µg /disc)	MIC (µg/ml)	MBC (µg/ml)
***Staphylococcus aureus* (MTCC 7443)**						
Hexane	13.0 ± 0.50	10.5 ± 0.50	8.6 ± 0.57	18.5 ± 0.50	500	1000
Chloroform	13.8 ± 0.28	10.5 ± 0.50	8.5 ± 0.50	15.6 ± 0.76	250	500
Ethyl acetate	16.1 ± 0.28	12.5 ± 0.50	10.3 ± 0.28	13.8 ± 0.57	250	500
Acetone	12.8 ± 0.50	11.1 ± 0.28	8.5 ± 0.57	15.0 ± 0.50	500	1000
Methanol	11.8 ± 0.76	9.5 ± 0.50	7.1 ± 0.28	18.5 ± 0.50	500	1000
***S. aureus* (MTCC 737)**						
Hexane	11.8 ± 0.76	10.5 ± 0.50	7.8 ± 0.76	7.3± 0.57	500	1000
Chloroform	13.0 ± 0.50	11.0 ± 0.50	7.6 ± 0.57	7.6± 0.76	250	500
Ethyl acetate	13.5 ± 0.50	10.5 ± 0.50	8.6 ± 0.57	8.5± 0.50	250	500
Acetone	12.8 ± 0.76	11.5 ± 0.50	7.8 ± 0.76	7.3 ± 0.57	500	1000
Methanol	10.6 ±0.28	8.6 ± 0.57	7.3 ± 0.28	7.6 ± 0.76	500	1000
MRSA 1						
Hexane	13.0 ± 0.50	11.1±0.28	8.5 ± 0.50	7.0 ± 0.50	500	1000
Chloroform	13.8 ± 0.28	9.8±0.57	7.8 ± 0.76	8.5 ± 0.50	250	500
Ethyl acetate	16.0 ± 0.50	12.5 ± 0.50	10.3 ± 0.57	7.5 ± 0.50	125	250
Acetone	12.8 ± 0.76	11.1 ± 0.28	7.6 ± 0.57	7.3 ± 0.57	500	1000
Methanol	12.5 ± 0.50	10.3 ± 0.57	7.5 ± 0.50	7.6 ± 0.76	500	1000
MRSA 2						
Hexane	12.1 ± 0.28	10.5 ± 0.50	7.6 ± 0.57	7.1 ± 0.28	500	1000
Chloroform	12.6 ± 0.76	11.5 ± 0.50	8.5 ± 0.50	7.3 ± 0.57	500	1000
Ethyl acetate	14.3 ± 0.57	11.3 ± 0.28	9.1 ± 0.76	8.3 ± 0.57	250	500
Acetone	12.8 ± 0.27	11.1 ± 0.28	7.6 ± 0.57	7.5 ± 0.50	500	1000
Methanol	12.5 ± 0.50	10.3 ± 0.28	7.5 ± 0.50	7 .8 ± 0.76	500	1000
MRSA 3						
Hexane	12.6 ± 0.28	10.5 ± 0.50	8.3 ± 0.28	7.3 ± 0.57	500	1000
Chloroform	11.8 ± 0.76	10.5± 0.50	8.5 ± 0.50	8.1 ± 0.28	500	1000
Ethyl acetate	12.8 ± 0.76	10.5± 0.50	8.5 ± 0.50	9.1 ± 0.28	250	500
Acetone	12.1 ± 0.28	10.5± 0.50	8.3 ± 0.57	8.0 ± 0.28	500	1000
Methanol	10.8 ± 0.28	9.5 ± 0.50	7.1 ± 0.28	7.3 ± 0.57	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation

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Table: 2 Antibacterial activity of *Stoechospermum marginatum* against bacterial strains

Bacteria strains / Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b] Concentration of the disc (µg/disc)					
	500	250	125	Methicillin (5µg /disc)	MIC (µg/ml)	MBC (µg/ml)
***Staphylococcus aureus* (MTCC 7443)**						
Hexane	13.8 ± 0.76	10.5 ± 0.50	8.8 ± 0.28	15.6 ± 0.76	250	500
Chloroform	14.3 ± 0.76	11.5 ± 0.50	8.1 ± 0.28	13.8 ± 0.57	125	250
Ethyl acetate	21.5 ± 0.50	16.0 ± 0.50	10.5 ± 0.50	15.0 ± 0.50	62.5	125
Acetone	13.5 ± 0.50	9.5 ± 0.50	8.0 ± 0.50	18.5 ± 0.50	250	500
Methanol	12.8 ± 0.76	10.0 ± 0.50	8.0 ± 0.50	13.8 ± 0.57	250	500
***S. aureus* (MTCC 737)**						
Hexane	13.5 ± 0.50	9.5 ± 0.50	8.5 ± 0.50	8.0 ± 0.50	250	500
Chloroform	15.0 ± 0.50	12.1 ± 0.28	8.6 ± 0.28	7.3 ± 0.57	250	500
Ethyl acetate	20.8 ± 0.76	16.0 ± 0.50	11.0 ± 0.50	7.6 ± 0.76	62.5	125
Acetone	13.8 ± 0.76	10.3 ± 0.57	7.8 ± 0.76	7 .6 ± 0.76	250	500
Methanol	12.8 ± 0.76	11.1 ± 0.28	8.5 ± 0.50	8.5 ± 0.50	500	1000
MRSA 1						
Hexane	14.6 ± 0.76	11.8 ± 0.28	8. 0 ± 0.50	7.5 ± 0.50	250	500
Chloroform	16.5 ± 0.50	12.0 ± 0.50	8.5 ± 0.50	7.3 ± 0.57	125	250
Ethyl acetate	19.5 ± 0.50	14.5 ± 0.50	10.8 ± 0.28	8.5 ± 0.50	62.5	125
Acetone	13.8 ± 0.28	10.5 ± 0.50	7.5 ± 0.50	7.1 ± 0.28	250	500
Methanol	12.5 ± 0.50	10.8 ± 0.28	7.5 ± 0.50	7.6 ± 0.76	250	500
MRSA 2						
Hexane	13.5 ± 0.50	10.6 ± 0.76	7.6 ± 0.57	8.1 ± 0.28	250	500
Chloroform	16.5 ± 0.50	11.8 ± 0.28	8.6 ± 0.57	7.1 ± 0.28	250	500
Ethyl acetate	18.8 ± 0.28	14.3 ± 0.57	10.5 ± 0.50	8.3 ± 0.57	125	250
Acetone	12.6 ± 0.57	10.5 ± 0.50	8.8 ± 0.76	7.5 ± 0.50	250	500
Methanol	11.5 ± 0.50	10.1 ± 0.28	8.3 ± 0.57	7 .8 ± 0.76	500	1000
MRSA 3						
Hexane	13.8 ± 0.28	11.6 ± 0.57	7.6 ± 0.57	7.3 ± 0.57	250	500
Chloroform	15.6 ± 0.57	10.6 ± 0.57	8.1 ± 0.28	8.3 ± 0.28	250	500
Ethyl acetate	19.5 ± 050	14.3 ± 0.57	10.3 ± 0.57	7.1 ± 0.28	62.5	125
Acetone	12.6 ± 0.57	11.6 ± 0.57	7.8 ± 0.76	7.8 ± 0.28	250	500
Methanol	10.6 ± 0.57	9.5 ± 0.50	7.8 ± 0.76	7.0 ± 0.50	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation

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Table: 3 Antibacterial activity of *Gracilaria edulis* against bacterial strains

Bacteria strains / Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	500	250	125	Methicillin (5µg /disc)	MIC (µg/ml)	MBC (µg/ml)
***Staphylococcus aureus* (MTCC 7443)**						
Hexane	13.1 ± 0.28	11.0 ± 0.50	7.3 ± 0.57	15.0 ± 0.50	500	1000
Chloroform	14.0 ± 0.50	11.0 ± 0.50	7.8 ± 0.28	18.5 ± 0.50	250	500
Ethyl acetate	16.6 ± 0.28	12.0 ± 0.50	9.0 ± 0.50	15.6 ± 0.76	125	250
Acetone	13.1 ± 0.28	9.1 ± 0.28	7.5 ± 0.50	13.8 ± 0.57	500	1000
Methanol	11.8 ± 0.76	10.5 ± 0.50	7.1 ± 0.28	15.0 ± 0.50	500	1000
***S. aureus* (MTCC 737)**						
Hexane	12.0 ± 0.50	10.0 ± 0.50	7.3 ± 0.57	8.0 ± 0.50	500	1000
Chloroform	14.0 ± 0.50	11.0 ± 0.50	9.1 ± 0.28	7.3± 0.57	250	500
Ethyl acetate	15.6 ± 0.28	11.6 ± 0.76	10.0 ± 0.28	7.6 ± 0.76	125	500
Acetone	12.5 ± 0.50	10.0 ± 0.50	7.6 ± 0.76	7.5 ± 0.50	500	1000
Methanol	11.6 ± 0.28	10.0 ± 0.50	7.1 ± 0.28	8.5 ± 0.50	500	1000
MRSA 1						
Hexane	13.0 ± 0.50	10.5 ± 0.50	8. 1 ± 0.28	7.1 ± 0.28	500	1000
Chloroform	13.1 ± 0.28	10.8 ± 0.57	8.1 ± 0.28	7.5 ± 0.50	250	500
Ethyl acetate	14.1 ± 0.28	11.5 ± 0.50	8.5 ± 0.50	7.3 ± 0.57	125	250
Acetone	12.3 ± 0.57	10.0 ± 0.76	7.3 ± 0.57	7.1 ± 0.28	500	1000
Methanol	12.0 ± 0.50	9.0 ± 0.50	7.1 ± 0.28	7.6 ± 0.76	500	1000
MRSA 2						
Hexane	11.5 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	8.1 ± 0.28	500	1000
Chloroform	12.1 ± 0.28	10.5 ± 0.50	7.3 ± 0.57	7.1 ± 0.28	250	500
Ethyl acetate	15.3 ± 0.57	11.5 ± 0.50	9.1 ± 0.28	7.3 ± 0.57	125	500
Acetone	12.6 ± 0.76	9.5 ± 0.50	7.5 ± 0.50	8.5 ± 0.50	500	1000
Methanol	12.0 ± 0.50	10.5 ± 0.50	7.5 ± 0.50	7 .8 ± 0.76	500	1000
MRSA 3						
Hexane	12.0 ± 0.50	10.5 ± 0.50	8.1 ± 0.28	7.3 ± 0.57	500	1000
Chloroform	12.5 ± 0.50	10.5 ± 0.50	7.5 ± 0.50	8.3 ± 0.28	500	1000
Ethyl acetate	15.5 ± 0.50	11.0 ± 0.50	8.1 ± 0.28	7.1 ± 0.28	125	500
Acetone	11.1 ± 0.28	10.5 ± 0.50	7.3 ± 0.57	8.5 ± 0.50	500	1000
Methanol	10.1 ± 0.28	9.0 ± 0.50	7.1 ± 0.28	7.3 ± 0.76	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation

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Table: 4 Antibacterial activity of *Gracilaria verrucosa* against bacterial strains

Bacteria strains / Seaweed extracts prepared with different solvents	Mean zone of inhibition[a] (mm)[b]					
	Concentration of the disc (µg/disc)					
	500	250	125	Methicillin (5µg /disc)	MIC (µg/ml)	MFC (µg/ml)
***Staphylococcus aureus* (MTCC 7443)**						
Hexane	13.0 ± 0.50	10.5 ± 0.50	7.3 ± 0.57	13.8 ± 0.57	250	500
Chloroform	13.1 ± 0.28	11.0 ± 0.50	7.8 ± 0.76	15.0 ± 0.50	250	500
Ethyl acetate	16.0 ± 0.50	12.0 ± 0.50	9.1 ± 0.28	18.5 ± 0.50	125	500
Acetone	13.3 ± 0.76	10.6 ± 0.28	7.8 ± 0.76	15.6 ± 0.76	500	1000
Methanol	12.3 ± 0.57	9.5 ± 0.50	7.1 ± 0.28	15.0 ± 0.50	500	1000
***S. aureus* (MTCC 737)**						
Hexane	12.1 ± 0.28	10.1 ± 0.28	7.3 ± 0.57	8.5 ± 0.50	500	1000
Chloroform	13.1 ± 0.28	11.0 ± 0.50	7.5 ± 0.50	8.0 ± 0.28	250	500
Ethyl acetate	14.6 ± 0.76	12.0 ± 0.50	8.8 ± 0.28	7.3 ± 0.57	125	250
Acetone	11.8 ± 0.76	9.1 ± 0.28	7.1 ± 0.28	7 .6 ± 0.76	500	1000
Methanol	11.1 ± 0.28	9.1 ± 0.28	7.3 ± 0.57	7.5 ± 0.50	500	1000
MRSA 1						
Hexane	13.1 ± 0.28	10.1 ± 0.28	7. 5 ± 0.50	7.5 ± 0.50	250	500
Chloroform	14.1 ± 0.28	10.5 ± 0.50	7.8 ± 0.28	7.0 ± 0.50	250	500
Ethyl acetate	15.0 ± 0.50	11.5 ± 0.50	8.5 ± 0.50	7.3 ± 0.57	125	250
Acetone	12.8 ± 0.28	10.6 ± 0.28	8.3 ± 0.57	8.5 ± 0.50	500	1000
Methanol	12.3 ± 0.57	9.6 ± 0.57	7.5 ± 0.50	7.6 ± 0.76	500	1000
MRSA 2						
Hexane	12.1 ± 0.28	10.5 ± 0.50	7.3 ± 0.57	8.1 ± 0.28	500	1000
Chloroform	13.5 ± 0.50	11.5 ± 0.50	8.3 ± 0.57	7.3 ± 0.57	250	500
Ethyl acetate	15.1 ± 0.57	14.0 ± 0.50	9.5 ± 0.50	7.5 ± 0.50	125	250
Acetone	12.0 ± 0.50	10.0 ± 0.50	8.0 ± 0.50	8.5 ± 0.50	500	1000
Methanol	11.1 ± 0.28	9.1 ± 0.28	7.3 ± 0.57	7 .8 ± 0.76	500	1000
MRSA 3						
Hexane	12.5 ± 0.50	11.1 ± 0.28	7.5 ± 0.50	8.3 ± 0.57	250	500
Chloroform	14.3 ± 0.57	10.5 ± 0.50	8.1 ± 0.28	7.3 ± 0.28	250	500
Ethyl acetate	15.0 ± 0.50	13.5 ± 0.50	10.3 ± 0.57	7.1 ± 0.28	125	250
Acetone	12.3 ± 0.57	11.1 ± 0.28	7.5 ± 0.50	7.5 ± 0.50	500	1000
Methanol	10.5 ± 0.50	9.3 ± 0.57	7.5 ± 0.50	7.3 ± 0.57	500	1000

[a]-diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]-mean of three assays; ± - standard deviation

DISCUSSION

In the present study, the ethyl acetate extract of brown algae showed higher antibacterial activity than red algae. Caccamese *et al.* [22] have reported that brown algal extracts showed higher activity than the extracts of red algae which was in accordance with the present results. In contrast, the red alga showed higher activity than the brown algae and green alga [23]. But variation in antibacterial activity may be due to the method of extraction, solvents used in extraction and season at which samples were collected [24]. Karthikaidevi *et al.* [25] reported that strong activities related to brown algae may be due to the phenolic compounds such as phlorotannins, eckol and eckol - related compounds that have strong bactericidal activity [26]. Plouguerne *et al.* [27] reported that the production of phenolic content of *Sargassum* species increased with the increased exposure to solar radiation in order to protect them from the UV radiation.

In this study, the different solvents *viz.*, hexane, chloroform, ethyl acetate, acetone and methanol extracts of *S. marginatum* followed by *G. edulis*, *G. verrucosa* and *S. wightii* have possessed antibacterial activity against all bacterial strains tested. In this study, ethyl acetate extract of *S. marginatum* showed the highest anti MRSA activity with a mean zone of inhibition of 21.5 mm against *S. aureus* (MTCC7443). The lowest MIC (62.5µg/ml) and MBC (125µg/ml) values of the ethyl acetate extracts of *S. marginatum* against *S. aureus* (737 & 7443), MRSA1 and MRSA3 were recorded. Thillairajasekar *et al.* [28] reported that the ethyl acetate extract of *Ulva lactuca* and *G. verrucosa* showed the highest antimicrobial activity against *E. coli*, *K. pneumoniae*, MRSA and *B. subtilis* and also identified the presence of myristic and palmitic acid, linoleic acid, oleic acid, lauric, stearic and myristic acid, from ethylacetate extracts. Salem *et al*. [29] reported that higher antibacterial activity was recorded for the ethyl acetate extracts of *Caulerpa racemosa*, *Sargassum dentifolium*, *Padina gymnospora* and methanolic extracts of *Sargassum hystrix*, *C. racemosa*, *C. fragile*, *S. dentifolium* and *Cystoseria myrica*. These results, contrast with the study of Lavanya and Veerappan [30] who reported that the methanol, chloroform, ethyl acetate and aqueous extracts of *Codium decorticatum*, *Caulerpa* scalpelliformis, *Gracilaria crassa*, *Acanthophora* spicifera, *S. wightii* and *Turbinaria conoides* were more active than the acetone, diethyl ether and hexane extracts against the bacterial pathogens.

In the present study, the ethyl acetate extracts of *S. marginatum*, *S. wightii*, *G. edulis*, and *G. verrucosa* possessed the antibacterial activity due to the presence of phytochemicals, terpenoids, tannins, phenolic compounds, and steroids. Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [31]. Zubia *et al.* [32] reported that great variation observed in the potential antimicrobial components in seaweeds could be due to the external environmental factors such as herbivory, light, depth, salinity and nutrients of their growing environment. Steroid glycosides are a class of widespread natural products having either terrestrial or marine origins. Several cardiac glycosides are used therapeutically in the treatment of cardiac failure and arrhytmias, and many glycoside compounds, belonging to other structural groups, cytotoxic, antimicrobial, hypocholesterolemic and other biological activities [33]. Tannins are well known to possess general antimicrobial properties [34].

In the present study, the highest activity of ethyl acetate extracts of *S. marginatum* followed by *G. edulis*, *G. verrucosa* and *S. wightii* extracts against methicillin resistant *S. aureus*. Lee *et al.* [35] reported that the ethyl acetate - soluble fraction of *Ecklonia stolonifera* and *Ecklonia cava* exhibited the strongest anti-MRSA activity. Dieckol isolated from *Ecklonia stolonifera* and *Ecklonia cava* is a known antibacterial substance with activity against MRSA [36]. Rosaline *et al.* [37] reported that maximum activities were recorded in the brown algae, *S. wightii* against MRSA in acetone and ethyl acetate extracts when compared to other solvents.

Hornsey and Hide [38] reported that 151 species of marine algal crude extracts showed inhibitory activity against pathogenic bacteria. They also reported that *G. corticata* did not show antibacterial activity. Rao and Parekh [39] reported that the extracts of *Enteromorpha intestinalis* and *G. corticata* showed antibacterial activity. They found that the algae were active throughout the year with a peak during the winter season.

Brown algae belonging to order dictyotales, *S. marginatum* is very abundant rich in sulfated fucans, and known to possess spasmogenic activity [40]. A new spatane diterpene, 17, 18-epoxy, 5(R), 16-dihydroxyspat 13(14)-ene, has been isolated from a brown alga *S. marginatum* [41] and various bioactivities, including antibacterial and antifungal [42] were reported.

MRSA is resistant to β-lactams, amino glycosides, fluoroquinolones and macrolide. In this study three control drugs namely methicillin, oxacillin and vancomycin were used. Although vancomycin is recognized as the most effective antibacterial agent for the control of MRSA [43] it has ototoxic and nephrotoxic effects [44]. Although strategies have been proposed in an attempt to control spread [45], the

search for new ways to treat MRSA infections stimulates the investigation of natural compounds as an alternative treatment of these infections.

Finally it can be concluded that ethyl acetate extracts of *S. marginatum* was found to be the most effective antiMRSA agent. This study recommends that ethyl acetate extracts of *S. marginatum* can be used as an antibacterial substance for treating MRSA infections after further scientific validation.

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